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Study of the Multiple Endocrine Neoplasia Type 1, Growth Hormone-Releasing Hormone Receptor, Gsα, and Gi2α Genes in Isolated Familial Acromegaly*

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ABSTRACT

Familial acromegaly may occur as an isolated pituitary disorder or as a feature of hereditary syndromes, such as multiple endocrine neoplasia type 1 (MEN1) or the Carney complex. Herein, we characterized a newly identified kindred with isolated acromegaly and searched for germline mutation in genes that have been associated with endocrine tumors [*i.e. MEN1*, Gsα (*GNAS1*), and Gi2α (*GNAI2*)], as well as the GHRH receptor (GHRH-R) gene. Genomic DNA was used to amplify exons 2–10 of *MEN1*, followed by dideoxy fingerprinting mutation analysis and direct sequencing. The GHRH-R gene was analyzed via direct sequencing of PCR-amplified fragments representing the coding exons and intron-exon junctions. To exclude mutation at hot spot areas of *GNAS1* and *GNAI2*, exons 8 and 9 of *GNAS1* and exons 5 and 6 of *GNAI2* were amplified and screened for mutation via denaturing gradient gel electrophoresis. No mutations were detected in any of the four genes. The present data extend prior reports of the absence of mutation in *MEN1, GHRH-R,* and *GNAS1* and describe the first family with isolated acromegaly in which germline mutation in *GNAI2* has been searched. (*J Clin Endocrinol Metab* **86:** 542–544, 2001)

ACROMEGALY IS A syndrome caused by excessive secretion of GH usually due to a GH-producing pituitary adenoma, occurring mostly as a sporadic disease (1). However, acromegaly may occur as an uncommon feature of familial disorders such as multiple endocrine neoplasia type 1 (MEN1) or the Carney complex. Isolated familial acromegaly is a rare syndrome and has been reported in approximately 20 families (2–5). Given the low incidence of acromegaly in MEN1 (6) or in the Carney complex (7) and the high penetrance of tumors in other endocrine glands in these syndromes, the presence of two or more cases of acromegaly in a family without other manifestations of MEN1 or the Carney complex strongly suggests an inherited pituitary syndrome distinct from these two more common syndromes.

The molecular basis of pituitary adenoma formation is still unknown. It probably derives from monoclonal proliferation of pituitary cells. Multiple molecular events may occur during tumor development, particularly the activation of oncogenes and inactivation of tumor suppressor genes (8).

Sporadic pituitary tumors (9) and some sporadic somatotropinomas have been associated with loss of heterozygosity on chromosome 11 (LOH on 11q13) (10), the locus of the MEN1 gene. Deletions on chromosome 11q13 have been reported in somatotropinomas from three families with isolated acromegaly (2, 4). Further, Gadelha *et al.* (11) reported linkage of isolated familial somatotropinomas to chromosome 11q13. Considering the possible linkage of familial acromegaly with the *MEN1* region, it seems reasonable to search for mutation in this gene in familial somatotropinoma.

Activating mutations in G protein-coupled receptors genes, such as LH receptor, have been described as causing neoplasia (12). As GHRH receptor (GHRH-R) is involved in somatotroph proliferation and GH secretion, one may predict that activating mutation in its gene might lead to GH overproduction and acromegaly. Thus, a germline activating mutation of the GHRH-R gene is another possible cause of isolated familial acromegaly.

The heterotrimeric G proteins, composed of α-, β-, and γ-subunits, are linked to cell surface receptors, resulting in the generation of an intracellular second messenger. Mutations of the α-subunit genes of Gs (*GNAS1*) and Gi2 (*GNAI2*) are associated with human neoplasms (13). Somatic mutations of *GNAS1* are found in GH-secreting pituitary adenomas and in somatotropinomas from patients with McCune-Albright syndrome (13, 14). Point mutations in the α-subunit of the G_{i2} protein gene have also been detected in 3 of 22 nonfunctioning pituitary adenomas and

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in 1 of 32 corticotroph adenomas (15, 16), but to date have not been sought in somatotropinomas.

The purpose of the present study was thus to characterize a newly identified Brazilian kindred with isolated acromegaly and to search for germline mutation in *MEN1*, *GHRH-R*, *GNAS1*, and *GNAI2* in this family.

Case Reports

Case 1

A 24-yr-old woman was referred for clinical evaluation in 1982 because of headaches, galactorrhea, menstrual irregularities, and progressive enlargement of hands and feet. Physical examination revealed evident acromegalic facial and acral features, with a height of 169 cm. GH levels were 34 μg/L (normal value, <5 μg/L) at baseline and 64 μg/L after oral administration of 75 g glucose. She had elevated basal PRL levels (111 μg/L; normal values, 2.5–14.5 μg/L). Insulin-like growth factor I (IGF-I) could not be measured before surgery. Pneumoencephalography showed a sellar volume of 2120 mm^3 (compatible with macroadenoma). Transsphenoidal adenomectomy was performed. Pathology report confirmed the diagnosis of pituitary adenoma, but immunohistochemical staining was not performed. After surgery, the PRL level was 9.0 μg/L, but elevated levels of GH and IGF-I led to the administration of radiotherapy.

Case 2

In 1986 the index case's 29-yr-old brother complained of a 10-yr history of progressive enlargement of hands, feet, and mandible. Physical exam revealed clear acromegalic features with a height of 179 cm. Basal GH level was 45.9 μg/L and rose to 66.5 μg/L after oral glucose administration. The PRL level was 7.9 μg/L. Pituitary computed tomography and magnetic resonance imaging scans documented a macroadenoma ($2.3 \times 1.1 \times 1.5$ cm). Transsphenoidal adenomectomy was performed, and pituitary irradiation was given because of high postoperative serum GH and IGF-I levels.

In both patients repeated values of basal intact PTH, total serum calcium, alkaline phosphatase, fasting glucose and insulin, and calcium-stimulated gastrin levels were normal. Exams in 1999 confirmed the diagnosis of isolated acromegaly not related to other endocrine syndromes.

Their father was likely to be the gene carrier for this condition. He had acromegalic features, confirmed by family pictures and died of a nonrelated cause at the age of 40 yr without endocrine evaluation. Five other first degree family members were screened. Blood samples were drawn after an overnight fast, and the levels of GH, IGF-I, PRL, total serum calcium, alkaline phosphatase, intact PTH, gastrin, and fasting glucose and insulin were measured, with no abnormality detected. This family is not related to another Brazilian kindred with somatotropinoma (4).

Materials and Methods

Diagnostic criteria

The diagnostic criteria used for isolated familial acromegaly were two or more members with GH-secreting pituitary adenoma without extrapituitary endocrine tumor. This study was approved by the ethics committees of the University of Sao Paulo School of Medicine and University Hospital. All subjects gave written informed consent to participate.

DNA isolation

Genomic DNA was isolated from peripheral blood leukocytes using DNA Quick-Prep Mini Kit (Lifecodes Corp., Stamford, CT) according to the manufacturer's protocol. Tumor samples for DNA analysis were not available.

MEN1 gene analysis

Exons 2–10 of *MEN1* comprising the coding region and flanking exon-intron boundaries were amplified individually or in groups from genomic DNA using published primers (http://www.niddk.nih.gov). PCR conditions were previously reported (17). The dideoxy fingerprinting (ddF) method (18) was used for mutation screening. The primers used for the ddF reactions were end labeled with γ-^{33}P using the T4 Polynucleotide Kinase Kit (Promega Corp., Madison, WI). ddF reactions were electrophoresed on a nondenaturing gel [0.75 $\times$ MDE (FMC Bioproducts, Rockland, ME) in 0.5 $\times$ TBE] using a sequencing apparatus as previously described (17).

Purified PCR products (Wizard PCR Preps DNA Purification System, Promega Corp., Madison, WI) were also subjected to direct sequencing (AmpliCycle Sequencing Kit, Perkin-Elmer Corp., Branchburg, NJ) with the same primers used for the ddF reactions. The sequencing reactions were run on a 6% denaturing polyacrylamide gel and autoradiographed overnight.

GHRH receptor gene analysis

The entire coding region and the exon-intron boundaries of *GHRH-R* were analyzed from peripheral genomic DNA via direct sequencing of PCR-amplified fragments. Each of the 13 exons and intron-exons boundaries was amplified individually, with the exception of exons 2 and 3, which were amplified together because they are divided by a short intron. PCR conditions and primer sequences were previously published (19). PCR products were separated through agarose gel and isolated in low melting agarose. They were subjected to direct sequencing using the Thermo Sequenase Cycle Sequencing Kit (Amersham Pharmacia Biotech, Cleveland, OH).

G protein gene analysis

Selected fragments, including exons 8 and 9 of *GNAS1* and exons 5 and 6 of *GNAI2*, were amplified by PCR and screened for mutation via denaturing gradient gel electrophoresis (DGGE) as previously reported (20). A 40-nucleotide G-C-rich sequence was attached to the 5′-end of the downstream primer to enhance the sensitivity of DGGE screening (21).

Results

The diagnosis of acromegaly and screening for MEN1 and Carney complex were based on clinical and hormonal data of the index case, her brother, and their relatives.

MEN1 mutation was screened with the primer sets covering the entire coding region and splice junctions. The ddF method and direct sequencing of *MEN1* did not reveal any mutation in the two affected subjects. A common silent *MEN1* polymorphism in codon 418 with a sequence change from GAC to GAT, both coding for aspartic acid, was found in both siblings (18).

The entire coding sequence and the intron-exon boundaries of *GHRH-R* were subjected to direct sequencing, and no changes were detected.

Mutations at the hot spot regions in exons 8 and 9 of *GNAS1* and in exons 5 and 6 of *GNAI2* were not detected when screened by DGGE in genomic DNA from the two affected patients.

Discussion

The absence of germline mutation in *MEN1* in our 2 affected siblings with acromegaly is in accordance with other studies involving 10 unrelated families with isolated acromegaly (3, 4). Somatic *MEN1* mutation has been excluded in another family with isolated acromegaly (5). The presence of LOH on 11q13 and the absence of *MEN1* mutation in some familial acromegaly cases raise the possibility that another tumor suppressor gene located in this region could be involved. In addition, *MEN1* has been analyzed in other familial pituitary tumors, such as isolated prolactinomas, and no mutation was found (22).

We also searched for germline mutation in other candidate genes, such as *GHRH-R*. To date, only inactivating mutations in *GHRH-R* have been reported in patients with familial isolated GH deficiency (19). Activating mutation in this gene would theoretically be able to cause somatotroph cells proliferation and GH hypersecretion, which might lead to acromegaly. Our present results extend the literature reports that a germline activating mutation in *GHRH-R* has not yet been found as a cause of familial acromegaly (4) and is not present in sporadic somatotropinomas (22a).

Naturally occurring (13) and experimentally induced (23) activating mutations of the α-subunit of G_s protein that lead to constitutive stimulation of adenylyl cyclase are associated with excessive secretion of GH as well as somatotroph proliferation. Mutations at hot spot regions of *GNAS1*, such as codons 201 and 227, were reported in human GH-secreting pituitary tumors (13). Considering these findings, we decided to search for germline mutation at hot spots areas of *GNAS1*, because tumor samples were not available, and no mutation was found. Somatic mutation at *GNAS1* has been sought by Ackermann *et al.* (5) in a family with isolated acromegaly, and direct sequencing of exons 7–13 of *GNAS1* detected no mutation. Taken together, these data suggest that *GNAS1* is not involved in the pathogenesis of these familial somatotropinoma cases.

G_{i2} protein inhibits adenylyl cyclase activity and has transforming activity in some cells in its mutated form (24). Amino acid homology alignment between the α-subunits of proteins G_{i2} and G_s revealed conservation of identical amino acids between codons 179 and 205 of *GNAI2* and codons 201 and 227 of *GNAS1*. Mutations in those homologous regions of *GNAI2* have been described in ovarian, adrenal, and pituitary tumors (13, 15, 16). As G_i protein binds to specific membrane receptor such as somatostatin, it seems reasonable that a mutation in the *GNAI2* could lead to excess secretion of GH. This idea was considered by searching for germline mutation at the homologous regions between α_s and α_{i2}-subunits of the G protein-coupled receptor. Our results show the absence of mutation in those regions, suggesting that hot spot areas of *GNAI2* may not be involved in the tumorigenesis of familial acromegaly. Although we have screened for *GNAS1* and *GNAI2* mutations using DGGE, this method has been documented to present sensitivity and specificity as high as 96% for detecting point mutations (25).

In conclusion, we extend prior reports of the absence of mutation in *MEN1, GHRH-R*, and *GNAS1* in families with isolated acromegaly and report for the first time the absence of mutation at hot spot areas of *GNAI2* in one family with this syndrome. Further studies of *GNAI2* in this entity should strengthen our conclusions.

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